生薬学雑誌 68 (1), 23-24 (2014)

Studies on the Constituents of the Leaves of *Odontioda* Marie Noel 'Velano'

Ryuichiro Suzuki, Setsuji Tomoeda, Mai Todoroki, Maki Horiguchi and Yoshiaki Shirataki*

Department of Pharmacognosy and Natural Medicines, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan

(Received July 16, 2013)

Three flavone glycosides (**1-3**) and two flavanone glycosides (**4, 5**) were isolated from the leaves of *Odontioda* Marie Noel 'Velano'. **1**, **3** and **4** indicated scavenging activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

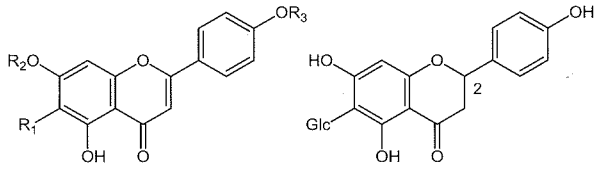
Keywords: *Odontioda* Marie Noel 'Velano', flavonoids, DPPH radical scavenging

Odontioda Marie Noel 'Velano' (Orchidaceae) is an intergeneric hybrid of *Odontoglossum* and *Cochilida*. *O.* Marie Noel 'Velano' is mainly cultivated as an ornamental plant.

We have previously reported the isolations of phenanthrenes, 5-hydroxy-2,3-dimethoxy-1,4-phenanthrenequinone and ephemeranthoquinone B from the bulb of this plant and their cytotoxicity against oral squamous cell carcinoma and leukenic cell lines. [1] There are no reports concerning studies on the constituents of this material besides ours. Therefore, we further investigated the constituents of this material. As a result, five known compounds were isolated; some of these isolates showed scavenging activity of the DPPH radical.

Leaves of *O.* Marie Noel 'Velano' (Orchidaceae) were supplied by the Orchid Garden Co., Ltd., Nagano Prefecture, Japan, in April 2009. This specimen was verified and identified by Mr. H. Sumiyoshi (Orchid Garden Co., Ltd.) and a voucher specimen (#20090617) was deposited in the Medicinal Plant Garden of Josai University.

The leaves of *O.* Marie Noel 'Velano' (3.6 kg) were extracted with MeOH in reflux. The MeOH extract was concentrated *in vacuo* to give corresponding extracts (492.2 g). Boiling water was added to the MeOH extract to remove the insoluble part. The aqueous soluble part was sequentially extracted with Et_2O, EtOAc, and *n*-BuOH. After removal of the solvent *in vacuo*, *n*-BuOH extract was passed through a Diaion HP-20 column, TOYOPEARL HW40C column, and Sephadex LH-20 column and purified by reversed-phase preparative HPLC to give linarin (**1**, 10.8 mg),[2] apigenin 6-*C*-neohesperidoside (**2**, 6.5 mg),[3] apigenin 7-*O*-rutinoside (**3**, 10 mg).[4] The EtOAc soluble fraction was chromatographed on a TOYOPEARL HW40C column and Sephadex LH-20 column, followed by reversed-phase HPLC to afford (*S*)-2-naringenin 6-*C*-β-glucopyranoside (**4**, 2.98 mg),[5] and (*R*)-2-naringenin 6-*C*-β-glucopyranoside (**5**, 3.29 mg).[6] These isolates were identified by comparison of spectroscopic data to literature values. The ^1H and ^{13}C NMR spectral data of **4** and **5** showed good resemblance; however, retention times of these compounds on HPLC analysis were slightly different. Furthermore, circular dichroism spectra of **4** and **5** showed the contrary Cotton effect at 290 nm.[7] The ^1H- and ^{13}C-NMR spectral data of isolates (**1-5**) are listed



	R_1	R_2	R_3
1	H	rut	Me
2	neoh	H	H
3	H	rut	H

4	2*S*
5	2*R*

Me: methyl, rut: rutinose, neoh: neohesperidose, Glc: glucose

Chart. Structures of isolated compounds **1** - **5**

Table 1. ^{1}H NMR (400 MHz) spectral data for **1-3** in DMSO-d_6 and **4**, **5** in MeOH-d_4.

	1	2	3		4	5
H-3	6.96 (1H, s)	6.66 (1H, s)	6.85 (1H, s)	H-2	5.33 (1H, dd, 3.0, 12.5)	5.33 (1H, dd, 2.9, 12.5)
H-6	6.45 (1H, d, 2.1)		6.45 (1H, d, 1.7)	H-3	2.71 (1H, dd, 3.0, 17.1)	2.71 (1H, dd, 2.9, 17.1)
H-8	6.79 (1H, d, 2.1)	6.36 (1H, s)	6.77 (1H, d, 1.7)	H-3	3.10 (1H, dd, 12.5, 17.1)	3.09 (1H, dd, 12.5, 17.1)
H-2', -6'	8.06 (2H, d, 9.0)	7.88 (2H, d, 8.6)	7.94 (2H, d, 8.8)	H-8	5.91 (1H, s)	5.90 (1H, s)
H-3', -5'	7.16 (2H, d, 9.0)	6.89 (2H, d, 8.6)	6.96 (2H, d, 8.8)	H-2', -6'	7.31 (2H, d, 8.6)	7.30 (2H, d, 8.5)
H-1''	5.07 (1H, d, 7.3)	4.65 (1H, d, 10.2)	5.06 (1H, d, 7.3)	H-3', -5'	6.81 (2H, d, 8.6)	6.81 (2H, d, 8.5)
H-2''	3.28 (1H, t, 8.3)	4.40 (1H, t, 8.9)	3.26 (1H, t, 8.4)	H-1''	4.78 (1H, d, 9.9)	4.78 (1H, d, 9.9)
H-3''	3.43 (1H, m)	3.38 (1H, m)	3.40 (1H, m)	H-2''	4.17 (1H, t, 9.1)	4.17 (1H, t, 8.6)
H-4''	3.15 (1H, m)	3.10 (1H, m)	3.14 (1H, m)	H-3''	3.44 (1H, m)	3.45 (1H, m)
H-5''	3.61 (1H, m)	3.16 (1H, m)	3.59 (1H, m)	H-4''	3.44 (1H, m)	3.45 (1H, m)
H-6''	3.43 (1H, m)	3.38 (1H, m)	3.46 (1H, m)	H-5''	3.36 (1H, m)	3.38 (1H, m)
H-6''	3.85 (1H, d-like, 7.6)	3.68 (1H, m)	3.84 (1H, d-like, 10.1)	H-6''	3.71 (1H, dd, 5.2, 12.0)	3.72 (1H, dd, 4.9, 12.0)
H-1'''	4.54 (1H, s-like)	5.01 (1H, br s)	4.54 (1H, s-like)	H-6''	3.85 (1H, dd, 2.1, 12.0)	3.85 (1H, dd, 2.0, 12.0)
H-2'''	3.70 (1H, s-like)	3.60 (1H, m)	3.65 (1H, s-like)			
H-3'''	3.48 (1H, m)	3.10 (1H, m)	3.46 (1H, m)			
H-4'''	3.15 (1H, t, 9.5)	2.93 (1H, t, 9.4)	3.14 (1H, t, 9.3)			
H-5'''	3.42 (1H, m)	2.45 (1H, m)	3.40 (1H, m)			
H-6'''	1.08 (3H, d, 6.2)	0.55 (3H, d, 5.9)	1.07 (3H, d, 6.2)			
5-OH	12.92 (1H, s)	13.52 (1H, s)	12.97 (1H, s)			
4'-OMe	3.86 (3H, s)					

Coupling constants (J in Hz) are given in parentheses.

Table 2. ^{13}C NMR (100 MHz) spectral data for **1-3** in DMSO-d_6 and **4**, **5** in MeOH-d_4.

	1	2	3	4	5
C-2	163.9	162.8	164.5	80.5	80.3
C-3	103.8	102.1	103.1	44.1	44.0
C-4	182.0	181.1	182.0	197.5	197.3
C-4a	105.5	102.2	105.4	102.8	102.8
C-5	161.2	161.8	161.6	164.7	164.6
C-6	99.9	108.8	99.9	106.5	106.5
C-7	162.4	162.6	162.9	170.0	170.0
C-8	94.7	93.5	94.8	97.3	97.5
C-8a	157.0	156.6	157.0	164.3	164.3
C-1'	122.7	120.7	120.9	131.3	131.3
C-2'	128.5	128.2	128.7	129.2	129.2
C-3'	114.7	116.2	116.2	116.5	116.5
C-4'	163.0	161.1	161.2	159.2	159.2
C-5'	114.7	116.2	116.2	116.5	116.5
C-6'	128.5	128.2	128.7	129.1	129.1
C-1''	99.7	71.6	99.6	75.5	75.6
C-2''	73.1	74.7	73.1	72.7	72.6
C-3''	76.3	80.2	76.3	80.5	80.3
C-4''	69.6	71.1	69.6	72.0	71.9
C-5''	75.7	81.4	75.6	82.7	82.7
C-6''	66.1	61.8	66.1	63.1	63.0
C-1'''	100.7	100.8	100.7		
C-2'''	70.4	70.4	70.4		
C-3'''	70.7	70.7	70.8		
C-4'''	72.1	71.8	72.1		
C-5'''	68.3	68.3	68.4		
C-6'''	17.8	17.6	17.9		
4'-OMe	55.6				

All compounds reported here were isolated for the first time from *O.* Marie Noel 'Velano'. DPPH radical scavenging activities of isolates were examined according to published methods.[8, 9] **1**, **3** and **4** showed moderate scavenging activities toward the DPPH radical with 50% effective concentration (EC_{50}) values of 3.88, 3.07 and 2.47 mM, respectively. Trolox, used as a positive control, had an EC_{50} value of 0.13 mM.

References

1) Masuda Y., Suzuki R., Sakagami H., Umemura N., Shirataki Y., *Chem. Pharm. Bull.*, **60**, 1216 – 1219 (2012).

2) Shin K. H., Kang S. S., Seo E. A., Shin S. W., *Arch. Pharm. Res.*, **18**, 65 – 68 (1995).

3) Rayyan S., Fossen T., Nateland S. H., Andersen M. Ø., *Phytochem. Anal.*, **16**, 334 – 341 (2005).

4) Baris O., Karadayi M., Yanmis D., Guvenalp Z., Bal T., Gulluce M., *J. Food Sci.*, **76**, T212 – T217 (2011).

5) Abdel-Kader M. S., *J. Braz. Chem. Soc.*, **8**, 637-639 (1997).

6) Kashiha K., Tomooka N., Vaunghan D. A., Kaga A., Ono H., Kameyama M., Yoshida M., Jpn. Kokai Tokkyo Koho, JP 2003137895 A (2003).

7) Caccamese S., Manna L., Scivoli G., *Chirality*, **15**, 661-667 (2003).

8) Nakamura A., *Tokyo Kasei Gakuin Daigaku Kiyo*, **44**, 5-8 (2004).

9) Sadhu S. K., Okuyama E., Fujimoto H., Ishibashi M., *Chem. Pharm. Bull.*, **51**, 595-598 (2003).